Context-Dependency of Relations Between Cardiovascular Phenotypes and Genes Involved in Sodium Homeostasis: Findings from the European Project on Genes in Hypertension

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Abstract: Hypertension is a chronic age-related disorder, affecting nearly 20% of all adult Europeans. This disease entails debilitating cardiovascular complications and is the leading cause for drug prescriptions in Europeans older then 50 years. Intensive research over the past two decades failed so far to identify common genetic polymorphisms with major impact on blood pressure or associated cardiovascular phenotypes, suggesting that multiple genes each with a minor impact, along with gene-gene and gene-environment interactions play a role. The European Project on Genes in Hypertension (EPOGH) is a large-scale family-based study, in which participants from 7 different populations were phenotyped and genotyped according to standardised procedures. This review article summarizes the initial 5-year findings and puts these observations into perspective against other published studies. EPOGH demonstrated that phenotype-genotype relations strongly depend on host factors, such as gender and lifestyle, in particular salt intake as reflected by the 24-hour urinary excretion of sodium. EPOGH therefore highlights the concept that phenotype-genotype relations can only be studied within a defined ecogenetic context.

Key Words: Cardiovascular system, genetics, epidemiology, gene-environment interactions.

INTRODUCTION

Cardiovascular disorders are the direct cause of death of over 60% of Europeans. Hypertension, a chronic age-related disorder, affects nearly 20% of all adults, entails debilitating cardiovascular complications and is causally involved in nearly 70% of all strokes [1]. From the viewpoint of health economics, both in Europe and the United States, hypertension is the leading cause for drug prescriptions in patients older than 50 years [2].

Hypertension arises through the complex interaction between genetic, environmental and lifestyle factors [3]. Searches for causative variants in chromosome regions identified by linkage analysis have been successful for rare monogenic forms of hypertension or hypotension. Technological advances in high-throughput genomics and proteomics and molecular medicine led to the discovery of 17 human genes that cause Mendelian forms of blood pressure dysregulation [4]. However, monogenic forms of hypertension are extremely rare. By contrast, linkage studies have been much less successful in locating genetic variants that affect common complex disorders, because each variant individually contributes only modestly to risk of disease. Until recently, 18 whole-genome scans with focus on blood pressure or hypertension [5,6] have been done in sib-pairs or families of African, Asian, or Caucasian origin. These studies identified more than 30 loci distributed over almost all the chromosomes, which showed linkage with human hypertension [5,6]. However, few of these loci were replicated in at least 2 independent populations. Until now, none of these genome-wide scans led to the discovery of common genetic variants,

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which may have a large impact on blood pressure in the population at large. It is conceivable that such genes do not exist and that blood pressure is dependent on a mosaic of many loci, each with small influence or with a contribution differing according to sex, race, age, or lifestyle.

Another approach to identify genetic causes of disease is to search for association with specific candidate genes. More than 150 candidate genes have been studied in relation to hypertension and other cardiovascular phenotypes. Often, strong associations are reported that are not confirmed in subsequent studies. However, a negative finding or a minor genetic effect in a general population may become a major gene effect in a subgroup of people with the appropriate genetic and environmental background. This can occur because of the network of feedback mechanisms regulating complex traits. Hence if the effects of locus A are masked by the exposure to factor B, the power to detect locus A is likely to be reduced if one does not consider factor B in the analysis. Gene-environment and gene-gene interactions (biological) are currently a topic of great interest in the field of genetic epidemiology. Better accounting for these interactions probably holds promise for advancing our understanding of the pathophysiology of blood pressure and related cardiovascular phenotypes.

The European Project on Genes in Hypertension (EPOGH) is a European family-based epidemiological survey with as objectives: (1) to search for interactions between genes and environment in the pathogenesis of hypertension and related intermediate phenotypes; (2) to produce a representative population-based database of complex cardiovascular phenotypes in relation to multiple genotypes and environmental factors. In this review, based on data from EPOGH, we will focus on the empirical observations of the interactions between different cardiovascular phenotypes, the genes encoding various components of the renin-angiotensin-aldosterone system (RAAS) and the adducin cytoskeleton, and sodium excretion as index of salt intake. The hypothesis of effect modification of the aforementioned genes by sodium intake was made *'a priory'* for the following reasons: (1) RAAS and adducin play a key role in sodium homeostasis [7,8]; (2) and *via* various mechanisms a high sodium might affect blood pressure, sympathetic tone, promote cardiac growth and change wall properties of the large arteries [9-11].

BLOOD PRESSURE, ENDOGENOUS OUABAIN AND ADDUCIN

Many genetic [8,12] and lifestyle [13] factors potentially interfere with the sodium-potassium pump, which plays an important role in sodium homeostasis and blood pressure regulation. For example, substitution of glycine by tryptophan at position 460 of the α-subunit of the heterodimeric cytoskeleton protein adducin (Gly460Trp) leads to increased sodium-potassium pump activity [12] and induces sodium retention [14]. Endogenous ouabain is a steroid hormone, which is released from the hypothalamus and the adrenal gland [15,16]. It behaves as a modulator of the sodium-potassium pump and exerts direct actions on the vasculature, the heart and tubular sodium reabsorption [15,16]. We therefore investigated the plasma concentration of endogenous ouabain in a Flemish population sample (n = 379) in relation to blood pressure, the Gly460Trp polymorphism of the α-adducin gene, and other determinants of sodium homeostasis [17]. Plasma ouabain (median, 140 pmol/L) correlated independently and positively with male gender (number of men, 182; $P = 0.002$), smoking (number of smokers, 116; $P = 0.05$), urinary potassium excretion (mean, 69 mmol/day; $P < 0.0001$), and the Trp mutation in the α-adducin gene (number of Trp carriers, 161; $P < 0.0001$). Both before and after adjustment for covariables, continuous as well as categorical analyses revealed a significant interaction ($P \leq 0.02$) between plasma ouabain and urinary sodium excretion (mean, 194 mmol/day) in relation to blood pressure (mean systolic/diastolic pressure, 123/76 mm Hg). In individuals with plasma ouabain values below the median, blood pressure increased ($P \leq 0.01$) by 2.2 mm Hg systolic and 1.4 mm Hg diastolic for each 50 mmol/day increment in urinary sodium excretion (Fig. **1**) [17]. No association between blood pressure and urinary sodium excretion was found when plasma ouabain exceeded the median.

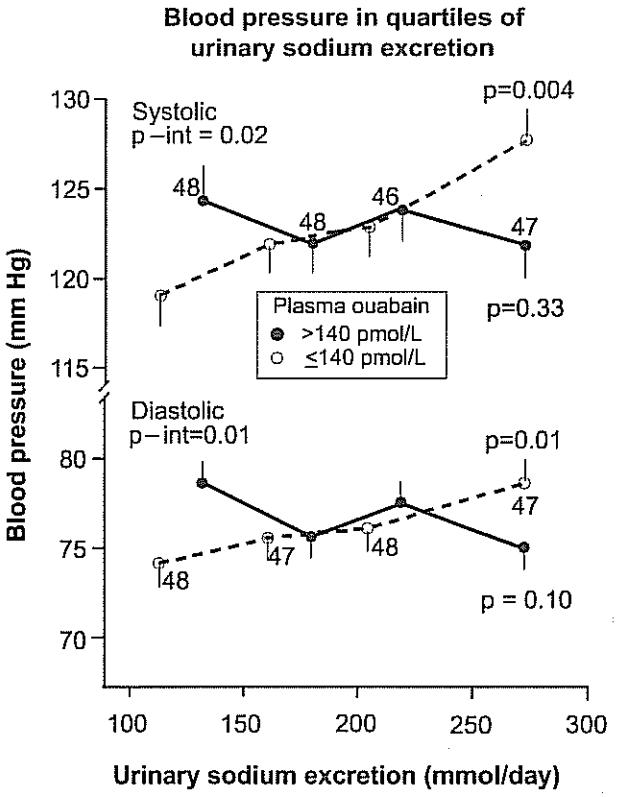

Fig. (1). Associations between blood pressure, plasma ouabain concentration and urinary sodium excretion [17]. Filled (plasma ouabain >140 pmol/L, median) and open symbols (≤140 pmol/L) represent the mean values of blood pressure in quartiles of urinary sodium excretion. The number of subjects in each quartile of urinary sodium excretion is shown alongside the symbols. Vertical lines denote SEs. Values were adjusted for gender, age, age^2, body mass index, smoking, alcohol intake, and use of antihypertensive drugs and/or oral contraceptives. Significance levels for trends (P) and interactions (P-*int*) are given.

One possible interpretation of our findings is that endogenous ouabain might play a central role in the homeo-

static regulation of blood pressure in response to changes in sodium intake. First, in sodium-deplete subjects, endogenous ouabain may enhance sodium retention. Several studies [18] have shown that very low subnanomolar concentrations of ouabain may stimulate – not inhibit – the renal sodium-potassium pump. At higher levels, during elevated salt intake, endogenous ouabain may act as a compensatory factor protecting against the development of sodium-sensitive high blood pressure, a hypothesis consistent with the commonly held view that this substance inhibits the sodium pump and promotes natriuresis [19]. Thus, plasma ouabain probably behaves as a blood pressure modulating factor, either inhibiting the pressor effect of an excessive salt intake or counteracting the depressor action of sodium depletion. Our study was observational in nature, not designed to reveal causality, and should therefore be considered as hypothesis generating. Nevertheless, if confirmed by experimental studies and other epidemiological observations, our findings might have clinical implications for the management of human hypertension and the prevention of cardiovascular disorders.

LEFT VENTRICULAR STRUCTURE

Condition such as chronically elevated blood pressure can cause cardiac remodelling in order to accommodate an increased load. The increase in left ventricular mass (LVM) is the first step towards the development of congestive heart failure and is a risk factor for the development of ischemic heart disease. The renin-angiotensin-aldosterone system not only regulates sodium and water homeostasis, but also emerges as a pivotal modulator of cardiovascular remodelling [20]. Its activity significantly modifies the development and regression of left ventricular hypertrophy.

Several actions of the RAAS are mediated by angiotensin II *via* stimulation of angiotensin II type 1 receptor (AT1). The angiotensin II type 2 receptor (AT2) receptor counterbalances the vasoconstrictor and antinatriuretic effects produced by angiotensin II *via* the AT1 receptor [21]. The AT2 receptor gene (*AGTR2*) maps to the X-chromosome [21]. Regulatory elements of the transcription of *AGTR2* are located in the promoter area and the first intron [22]. The *AGTR2* G1675A polymorphism, which is located in intron 1, is likely to be functional in that the G allele is associated with increased *AGTR2* transcription and therefore with increased expression of the AT2 receptor [22]. On the other hand, in animal experiments, dietary sodium depletion also enhanced the expression of AT2 receptors [23]. Thus, one might speculate that a pronounced expression of AT2 receptor in G allele carriers, particularly in sodium depletion, can exert protective effects. Indeed, in our study [24], we observed that in men the effect of the *AGTR2* G1675A polymorphism on LVM differed according to sodium excretion. In women, this gene-environment interaction did not reach statistical significance. Continuous analyses demonstrated that in male G allele carriers LVM index and left ventricular internal diameter, but not wall thickness, increased with higher sodium excretion. Further analyses involving only untreated men and dichotomized according to the median sodium excretion showed that when sodium excretion was less than 240 mmol/day (median), LVM index was lower in G than A allele carriers (Fig. **2**) [24].

Only few studies addressed the possible association of LVM with the *AGTR2* G1675A polymorphism in humans [25,26] . In accordance with our findings, the G allele frequency in these studies varied from 43% to 51%. In 120 men

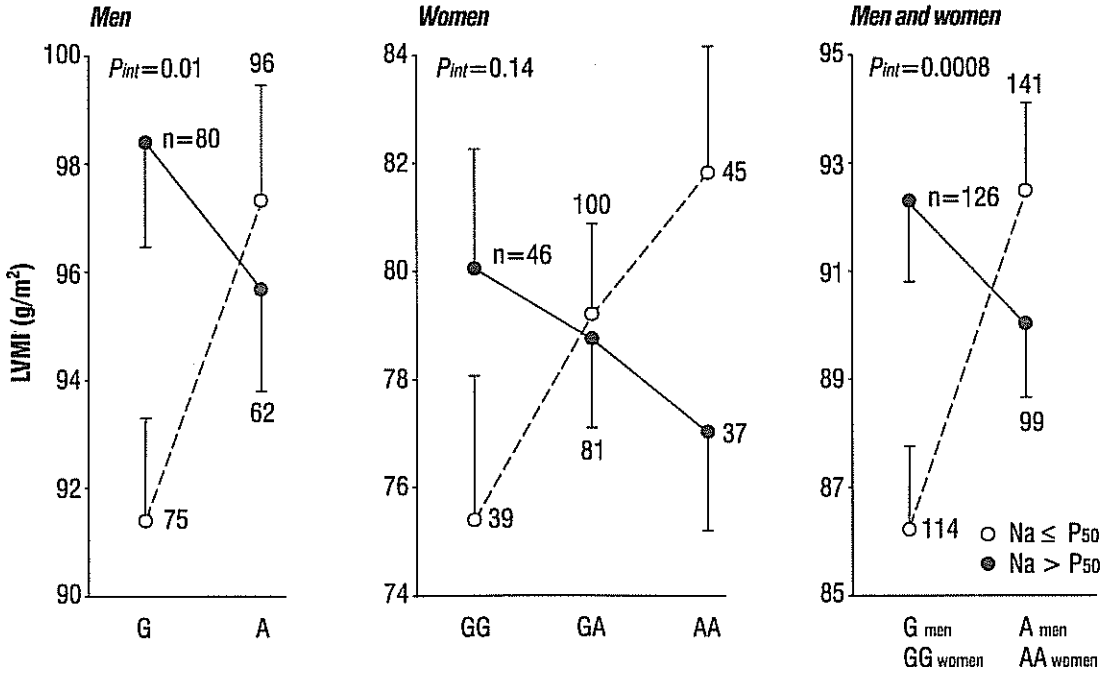

Fig. (2). LVMI in relation to the *AGTR2* polymorphism in untreated subjects [24]. Associations were plotted for 2 groups based on sex- and country-specific medians of sodium excretion. Test statistics for the interaction with sodium excretion, analysed as a continuous variable, were derived by generalized estimation equation (GEE). Adjustments included center, sex, age, body weight and height (not applicable to LVMI), waist-to-hip ratio, systolic blood pressure, current smoking, alcohol intake, and antihypertensive treatment.

with normal or mildly elevated blood pressure and mean age of 26 years, Schmieder *et al.* [25] found that hypertensive but not normotensive G allele carriers had a lower left ventricular mass than A allele carriers due to a reduced wall thickness. Herrmann *et al.* [26] observed a higher prevalence of the G allele in 55- to 74-year old Scottish men (n = 336) without electrocardiographic left ventricular hypertrophy enrolled in the GLAOLD study compared to those with hypertrophy. However, these researches could not confirm their findings using electrocardiographic and echocardiographic phenotyping in 629 men, 25- to 74-years old, investigated in the framework of the GLAECO study [26], even if the subjects older than 55 years were studied separately.

None of the previous studies in humans accounted for sodium intake. The hypothesis that the AT2-mediated effects of angiotensin II on LVM are modulated by sodium intake is plausible, although the exact mechanism remains to be elucidated. Indeed, in animal experiments, dietary sodium depletion enhanced the expression of AT2 receptors [23]. The latter may play a counter-regulatory role that opposes the sodium retaining effect of angiotensin II mediated *via* the AT1 receptor. Moreover, in sodium-depleted rodents, stimulation of the AT2 receptor produced natriuresis [27], whereas the opposite might occur in sodium-replete animals [28].

LVM is calculated from the left ventricular internal diameter and wall thickness. Left ventricular diameter, to some degree, reflects the circulating fluid volume, whereas wall thickness might be more indicative of processes confined to the myocardium itself. We found that the interaction between the G1675A polymorphism and sodium excretion in relation to LVM was mediated *via* left ventricular internal diameter. This suggests that renal AT2 receptors *via* their influence on sodium balance and the circulating plasma volume, might also contribute to the explanation of our findings. On the other hand, we also confirmed an independent effect of the angiotensin-converting enzyme (*ACE)* D/I genotype on LVM. As we noticed in a previous publication [29], this effect was mediated *via* wall thickness and it was also dependent on sodium intake. Thus, overall, our findings support the hypothesis that the *AGTR2* G1675A polymorphism independently influences LVM and that salt intake modulates this genetic effect. However, because of the exploratory nature of our analyses, which is inherent to all observational studies, our findings need replication in future human and experimental research.

HEART RATE VARIABILITY

Measurements of plasma catecholamines and norepinephrine spill-over, direct recordings from peripheral nerves and power spectral analysis of heart rate variability, and responses to pharmacological blockade suggest that the autonomic nervous system contributes to the development and maintenance of high blood pressure in human essential hypertension. Stimulation of the sympathetic nervous system and/or a decrease of parasympathetic activity may be involved in the development of essential hypertension. Sympathetic tone increases with stimulation of the RAAS and is under the influence of salt intake [30]. We therefore investigated whether polymorphisms in the genes encoding aldosterone synthase (*CYP11B2* C–344T) and the type-1 angiotensin II receptor (*AGTR1* A1166C) affect the autonomic modulation of heart rate variability at varying levels of salt intake [31]. We measured the low frequency (LF) and high frequency (HF) components of heart rate variability and their ratio (LF:HF) in the supine and standing positions in 1797 participants (401 families), randomly recruited from 6 European populations, whose average urinary sodium excretion ranged from 163 to 245 mmol/d. When we did not account for sodium excretion, neither the population-based nor the family-based analyses revealed any significant association between heart rate variability and the genetic polymorphisms. However, in subjects with sodium excretion below 190 mmol/day, supine heart rate, LF and LF:HF increased and HF decreased with the number of *CYP11B2* –344T alleles. The orthostatic changes in LF, HF and LF:HF were blunted in carriers of the *AGTR1* 1166C allele (Fig. 3). In subjects with sodium excretion above 190 mmol/d, these associations with the *CYP11B2* and *AGTR1* polymorphisms were non-significant or in the opposite direction, respectively (Fig. 3).

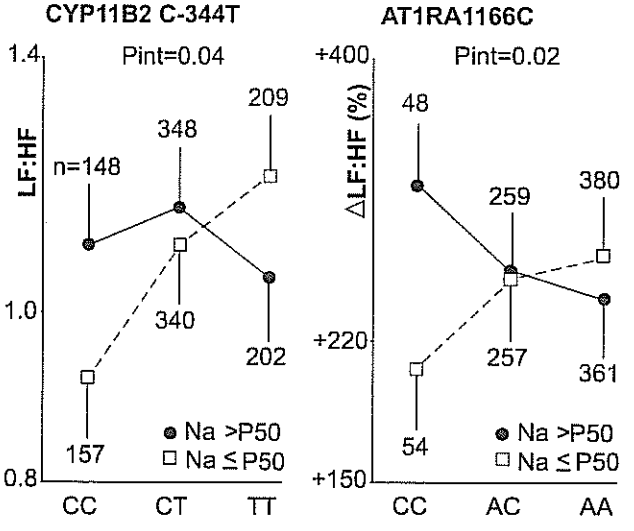

Fig. (3). Low-to-high frequency ratio in the supine position (LF:HF) and orthostatic change in this ratio expressed as a percentage of its value in the supine position (ΔLF:HF) in relation to the *CYP11B2* and *AT1R* polymorphisms, respectively [31]. Associations were plotted for 2 groups, based on country- and sex-specific medians of sodium excretion. The probability of the interaction (P_{int}) between genotype and sodium excretion analyzed as a continuous variable was derived by GEE and accounts for nonindependence within families and covariates.

Our findings [31], in keeping with other reports in the literature [32,33] , support the hypothesis that genetic polymorphisms and lifestyle factors leading to expansion of the circulating plasma volume might affect the autonomic nervous regulation of the cardiovascular system. An excessive salt intake might be associated with an expanded circulating plasma volume [34], which in turn might mask the genetic influence of the *CYP11B2* C–344T polymorphism on heart rate variability. Salt intake modulates the expression of AT1 receptors [35]. Depending on salt intake, the *AGTR1* 1166CC genotype may or may not be associated with a tendency for

volume expansion, which might explain the differential autonomic modulation of heart rate variability in CC homozygotes. Thus, the *CYP11B2* C–344T and *AGTR1* A1166C polymorphisms affect the autonomic modulation of heart rate variability in the supine and standing positions, respectively, but these genetic effects depend on sodium intake.

ARTERIAL WAVE REFLECTION

Angiotensin II and aldosterone also influence vascular remodelling in response to high blood pressure. In 622 EPOGH subjects from 3 European populations, we therefore investigated whether the *ACE* D/I and *CYP11B2* C–344T polymorphisms influence arterial wave reflections, a measure of vascular stiffness [36].

The peripheral and central augmentation indexes were significantly higher in *CYP11B2* –344C allele carriers than in –344T homozygotes in population-based (Fig. 4) and family-based analyses. However, this effect of the *CYP11B2* polymorphism only occurred in subjects with a higher than median urinary sodium excretion (210 mmol/day). The association between systolic augmentation and the *ACE* D/I polymorphism did not reach statistical significance [36].

Previous studies demonstrated that in humans a high salt intake is associated with increased arterial stiffness and vascular hypertrophy [11]. Furthermore, stroke-prone spontaneously hypertensive rats fed 0.9% NaCl in drinking water, compared to a control group given tap water, had increased expression of mRNA for *CYP11B2* in the arterial wall, but lower levels of circulating aldosterone [37]. Thus, an excessive salt intake might contribute to increased arterial stiffness by inappropriately sustaining the expression of the *CYP11B2* gene in the arterial wall, especially in –344C allele carriers.

CONCLUSIONS

Blood pressure and associated cardiovascular phenotypes are continuous traits, of which the genetic determination differs according to gender, age, lifestyle and various environmental factors [24,29,31,36] . Throughout life, genetically determined host factors continuously interact with environmental influences. Any resulting change in a given phenotype is initially counteracted by self-organizing homeostatic mechanisms, which encompass intracellular signaling, metabolic and hormonal regulation at the cell and the tissue level, as well as systemic feedback loops involving the whole body. Moreover, many genes may influence the same phenotype or a single mutation may be associated with grossly discordant phenotypes. Even in monogenic disorders with typical Mendelian inheritance [38], penetrance may vary according to race, age, dietary salt intake, or hormonal milieu. Mendelian principles or the biometrical assumptions underlying the decomposition of variance into genetic, environmental and random components, may not apply to multigenic quantitative traits, such as hypertension [3,39,40] . Along these lines, EPOGH for instance demonstrated that individuals with the same genetic predisposition had different left ventricular mass [24,29] , heart rate variability [31] or vascular stiffness [36], depending on whether they ate a high-sodium or a low-sodium diet. As exemplified by the present results, environmental factors modify the actions of genes. Thus, population studies, which take into account gene-

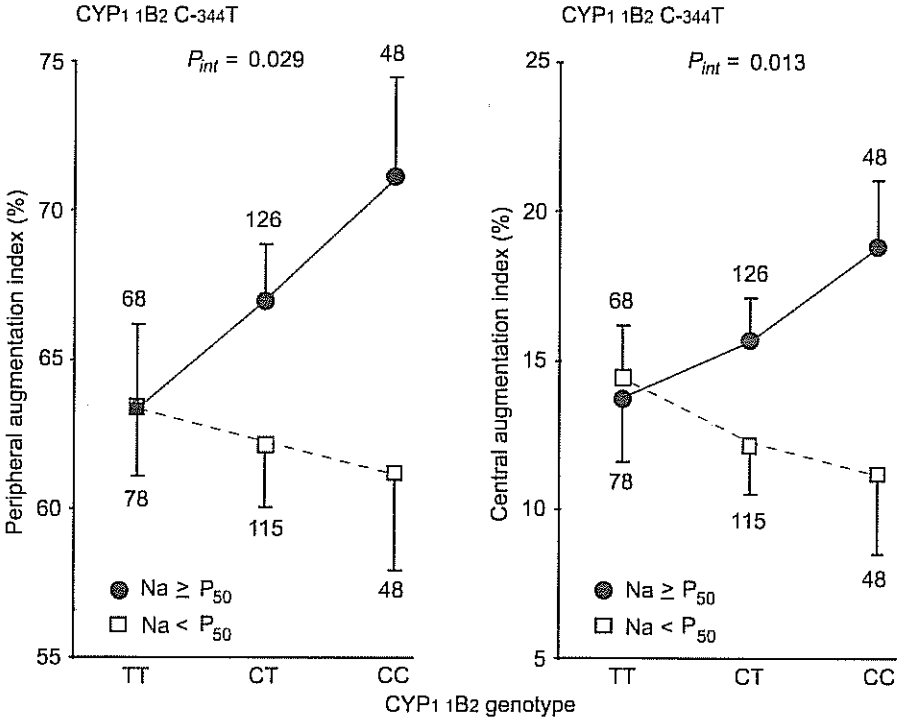

Fig. (4). Peripheral and central augmentation indexes by CYP11B2 genotype and median sodium excretion (210 mmol/day) in 483 untreated subjects [36]. Values are adjusted means ± SE. The significance of the genotype-by-sodium interaction (*Pint*) was derived from a GEE model, which included sodium excretion as a continuous variable and which accounted for clustering within families and significant covariates.

environment and gene-gene interactions, will increasingly be used to study complex cardiovascular phenotypes.

In conclusion, we suggest that genetic research should be based on a close integration of basic and clinical medicine. Basic scientists will continue to generate the physiological and pathophysiological knowledge to understand phenotype-genotype relations, and to generate new *a priori* hypotheses to be tested in human subjects. Conversely, epidemiological observations, unaccounted for by pathophysiological pathways and confirmed in independent population samples, must be further explored in animal experiments and genomic or proteomic research at the molecular level.

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APPENDIX

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